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## Immunological Reactions to Mycobacterial Proteins in the Spectrum of Leprosy

### TO THE EDITOR:

Leprosy, still a public health problem in many parts of the world, is a chronic mycobacterial disease produced by an intracellular parasite, *Mycobacterium leprae*, which multiplies mainly within tissue macrophages and Schwann cells in peripheral nerves. A good immunological response toward *M. leprae* depends on the development of a T-cell-mediated immune response.

It has not been established whether individual proteins are sufficient for the development of protective immunity in mycobacterial infections. Also, the availability of purified and well-characterized antigens is a prerequisite for studying the role of individual molecules in the pathogenesis of the disease.

We have previously studied the effect of soluble *M. leprae* extract on the proliferative response of peripheral blood lymphocytes from leprosy patients, their family members and other contacts. In tuberculoid patients and in contacts we detected differential reactivity to the various proteins isolated (\*).

Using molecular biology techniques and monoclonal antibodies, it has been possible to obtain individual proteins from mycobacterial antigens (<sup>1,2,6,8,15</sup>), which has been very helpful for understanding the biology of this microorganism and its relationship with the host.

In this study, we have compared cellular and humoral reactivity toward various whole, fractionated and recombinant mycobacterial antigens from *M. bovis* and *M. leprae* in patients from the outpatient clinic of the Instituto de Biomedicina, Caracas, Venezuela. These patients were clinically and histologically diagnosed according to Ridley and Jopling's classification (<sup>11</sup>). The group included 20 lepromatous (LL) and borderline lepromatous (BL) patients and 20 tuberculoid (TT) patients, as well as a group of 10 family and nonfamily Mitsuda-positive contacts. All LL and BL (85% males, 15% females) and TT (40% males, 60% females) patients were adults.

The antigens used for this study were: bacilli from experimentally infected armadillo lesions purified using Draper's method, BP (\*); soluble *M. leprae* extract

TABLE 3. ELISA, serum pools with mycobacterial recombinant antigens.

Patients	Recombinant proteins					
	70	65	36	28	18	10
Multibacillary (LL and BL)	0.453 <sup>a</sup> 0.641 <sup>b</sup>	0 0.107	0.221 0.736		0.317 0.866	1.399 1.264
Paucibacillary (TT)	ND <sup>c</sup> ND	0.030 0.045	ND ND		ND ND	ND ND
Contacts	0.140 0.291	0 0	ND ND		0.220 0.451	0 0

<sup>a</sup> OD values protein concentration 5 µg/ml.

<sup>b</sup> OD values protein concentration 20 µg/ml

<sup>c</sup> 2 µg/ml.

<sup>d</sup> 10 µg/ml.

\* ND = Not done.

Work done in an endemic region of Ethiopia with 24 tuberculoid and 18 lepromatous patients and 21 healthy contacts using 11 antigenic molecules including *M. leprae* heat-shock protein (hsp) 10 and hsp18 and hsp65 did not show any association between the antigen-specific cellular response and the status of the various patients and controls studied (13).

Concerning cellular immunity, it is evident that the complete *M. leprae* protein extract is more immunogenic than individual proteins; if we study the percentage of positivity in the lymphocyte transformation tests of paucibacillary patients and family or nonfamily contacts, it is clear that several proteins are undoubtedly involved in the immune response. The stimulation index of T lymphocytes from lepromatous patients toward MISA varied between 0.55, and 1.52; in tuberculoid patients the index varied between 1.72 and 10.02; in contacts, between 2.21 and 39.82.

**ELISA study.** IgG antibodies directed toward recombinant proteins were determined in an enzymatic assay (10). The values of circulating IgG antibodies directed toward complete extracts (cytosol and cell wall) and recombinants from *M. leprae* and *M. tuberculosis* were expressed in optical density (OD). Microtiter plates were sensitized with 5 µg/ml and 20 µg/ml recombinant antigens and 2 µg/ml and 10 µg/ml soluble *M. leprae* extract, 50 µl per well; one well was left with no antigen and two wells with antigen for each serum pool (LL and BL, T, and contacts) (14). Preliminary results using serum pools in the leprosy spectrum are shown in Table 3.

IgG antibodies responded in great portion to complex MISA and *M. tuberculosis* 10-kDa protein, and in decreasing order to *M. tuberculosis* 70, *M. leprae* 18, and *M. leprae* 36 kDa. With *M. bovis* 65 kDa it was not possible to obtain good detector groups studied. Other workers have shown that in the majority of leprosy patients and their contacts, IgG reacted to both of the 10-kDa proteins from *M. tuberculosis* and *M. bovis* (BCG) (?). Quantitative differences were found in this study using a pool of sera from patients and contacts are not clear. At present, we are developing sequential assays before, during and after multidrug therapy in individual patient's sera.

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